09/882,509

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=> d his

     (FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004
L1         40597 S STREPTOKINASE?
L2           362 S "S. EQUISIMILIS"
L3           103 S L1 AND L2
L4       6513995 S CLON? OR EXPRESS? OR RECOMBINANT
L5            74 S L3 AND L4
L6            29 DUP REM L5 (45 DUPLICATES REMOVED)
L7         44795 S INCLUSION (A) BOD?
L8             1 S L6 AND L7
L9            42 S "LAMBDAPR"
L10            0 S L2 AND L9
L11            0 S L1 AND L9
L12            0 S L9(W) LAMBDAPL
L13            0 S "LAMBDAPR-LAMBDAPL"
L14           35 DUP REM L9 (7 DUPLICATES REMOVED)
                 E KUPPUSAMY M/AU
L15           45 S E3-E6
                 E MOSUVAN M/AU
                 E MOSUVAN K/AU
                 E VELLIMEDU S/AU
                 E LAHIRI S/AU
L16         1594 S E3
                 E KRISHNA E/AU
L17           18 S E3-E7
L18         1612 S L16 OR L17
L19            0 S L1 AND L18
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 NEWS  3  JAN 27   Source of Registration (SR) information in REGISTRY updated
                   and searchable
 NEWS  4  JAN 27   A new search aid, the Company Name Thesaurus, available in
                   CA/CAplus
 NEWS  5  FEB 05   German (DE) application and patent publication number format
                   changes
 NEWS  6  MAR 03   MEDLINE and LMEDLINE reloaded
 NEWS  7  MAR 03   MEDLINE file segment of TOXCENTER reloaded
 NEWS  8  MAR 03   FRANCEPAT now available on STN
 NEWS  9  MAR 29   Pharmaceutical Substances (PS) now available on STN
 NEWS 10  MAR 29   WPIFV now available on STN
 NEWS 11  MAR 29   New monthly current-awareness alert (SDI) frequency in RAPRA
 NEWS 12  APR 26   PROMT: New display field available
 NEWS 13  APR 26   IFIPAT/IFIUDB/IFICDB: New super search and display field
                   available
 NEWS 14  APR 26   LITALERT now available on STN
 NEWS 15  APR 27   NLDB: New search and display fields available
 NEWS 16  May 10   PROUSDDR now available on STN
 NEWS 17  May 10   PROUSDDR: One FREE connect hour, per account, in both May
                   and June 2004

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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
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                                                   ENTRY    SESSION
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FILE 'MEDLINE' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'EMBASE' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'BIOSIS' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'BIOTECHDS' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'SCISEARCH' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'HCAPLUS' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'NTIS' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004


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=> s streptokinase2
2 IS NOT A RECOGNIZED COMMAND
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=> s streptokinase?
L1       40597 STREPTOKINASE?

=> s "S. equisimilis"
L2         362 "S. EQUISIMILIS"

=> s l1 and l2
L3         103 L1 AND L2

=> s clon? or express? or recombinant
   5 FILES SEARCHED...
L4     6513995 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l4
L5          74 L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6           29 DUP REM L5 (45 DUPLICATES REMOVED)

=> d 1-29 ibib ab

L6   ANSWER 1 OF 29  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                              DUPLICATE 1
ACCESSION NUMBER:   2003404343  EMBASE
TITLE:              Expression of streptodornase by use of
                    streptokinase promoter in Streptococcus equismilis
                    H46A.
```

AUTHOR: Sohn H.-J.; Chin J.; Kim I.-C.; Bai S.; Lee H.B.
CORPORATE SOURCE: H.B. Lee, Department of Biological Sciences, Chonnam National University, Gwangju 500-757, Korea, Republic of. blaise@chonnam.chonnam.ac.kr
SOURCE: Korean Journal of Microbiology and Biotechnology, (2003) 31/3 (307-310).
Refs: 18
ISSN: 1598-642X CODEN: HMHAAS
COUNTRY: Korea, Republic of
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: Korean
SUMMARY LANGUAGE: English

AB A gene encoding streptodornase(sdc) from Streptococcus equisimilis H46A was **expressed** in **S. equisimilis** H46A sdc(-) under the control of the **streptokinase** gene promoter. Secretion of the streptodornase was directed by the signal sequences of **streptokinase** or streptodornase. The **expressed** streptodornase activity from **S. equisimilis** H46A sdc(-) transformant with **streptokinase** promoter - streptodornase coding sequence fusion vector was 2.3 fold higher than that from wild type. Construct of signal sequence region replaced by **streptokinase** ones was similarly **expressed** as a wild type. But constructs of skc or lrp core regions of **streptokinase** promoter streptodornase fusion were similarly **expressed** as in sdc(-) mutant. In conclusion, improved **expression** of streptodornase by use of **streptokinase** promoter required the full length of promoter.

L6 ANSWER 2 OF 29 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2000038313 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10569766
TITLE: **Cloning**, **expression**, sequence analysis, and characterization of **streptokinases** secreted by porcine and equine isolates of Streptococcus equisimilis.
AUTHOR: Caballero A R; Lottenberg R; Johnston K H
CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.
CONTRACT NUMBER: R01DK45014 (NIDDK)
SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (Streptococcus equi, **S. equisimilis**, and S. zooepidemicus) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from **S. equisimilis** strains which activated either equine or porcine plasminogen were **cloned**, sequenced, and **expressed** in Escherichia coli. The **streptokinase** secreted by the equine isolate had little similarity to any known **streptokinases** secreted by either human or porcine isolates. The **streptokinase** secreted by the porcine isolate had limited structural and functional similarities to **streptokinases** secreted by human isolates. Plasminogen activation

studies with immobilized (His)(6)-tagged **recombinant** **streptokinases** indicated that these **recombinant** **streptokinases** interacted with plasminogen in a manner similar to that observed when **streptokinase** and plasminogen interact in the fluid phase. Analysis of the cleavage products of the **streptokinase**-plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which **streptokinase** was cleaved to form altered **streptokinase** (Sk*) was also determined. This study confirmed not only the presence of **streptokinases** in nonhuman **S. equisimilis** isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L6 ANSWER 3 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
ACCESSION NUMBER: 2000:96119 BIOSIS
DOCUMENT NUMBER: PREV200000096119
TITLE: Two **streptokinase** genes are **expressed** with different solubility in Escherichia coli W3110.
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002

AB The **streptokinase** (SK) gene from **S. equisimilis** H46A (ATCC 12449) was **cloned** in E. coli W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from **S. equisimilis** (ATCC 9542) which had been **cloned** in E. coli W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of inclusion bodies.

L6 ANSWER 4 OF 29 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999150235 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10024545
TITLE: Purification and **cloning** of a **streptokinase** from Streptococcus uberis.
AUTHOR: Johnsen L B; Poulsen K; Kilian M; Petersen T E
CORPORATE SOURCE: Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus C, Denmark.
SOURCE: Infection and immunity, (1999 Mar) 67 (3) 1072-8.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ131604; GENBANK-AJ131605; GENBANK-AJ131631
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990326
Last Updated on STN: 19990326
Entered Medline: 19990312

AB A bovine plasminogen activator was purified from the culture supernatant

of the bovine pathogen Streptococcus uberis NCTC 3858. After the final reverse-phase high-performance liquid chromatography step a single protein with a molecular mass of 32 kDa was detected in the active fraction. A partial peptide map was established, and degenerate primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse PCR was subsequently used for obtaining the full-length gene. The S. uberis plasminogen activator gene (skc) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In an amino acid sequence comparison the **cloned** activator showed an identity of approximately 26% to the **streptokinases** isolated from Streptococcus equisimilis and Streptococcus pyogenes. Interestingly, the activator from S. uberis was found to lack the C-terminal domain possessed by the **streptokinase** from **S. equisimilis**. This is apparently a general feature of the **streptokinases** of this species; biochemical and genetic analysis of 10 additional strains of S. uberis revealed that 9 of these were highly similar to strain NCTC 3858. Sequencing of the skc gene from three of these strains indicated that the amino acid sequence of the protein is highly conserved within the species.

L6 ANSWER 5 OF 29 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1999-03949 BIOTECHDS
TITLE: Purification and **cloning** of a **streptokinase** from Streptococcus uberis; cattle plasminogen-activator purification and characterization
AUTHOR: Johnson L B; Poulsen K; Kilian M; *Petersen T E
CORPORATE SOURCE: Univ.Aarhus
LOCATION: Protein Chemistry Laboratory, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark.
Email: tep@mbio.aau.dk
SOURCE: Infect.Immun.; (1999) 67, 3, 1072-78
CODEN: INFIBR
ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A cattle plasminogen-activator was purified from the culture supernatant of Streptococcus uberis NTCTC 3858. After the final reverse-phase HPLC step, a single protein with a mol.weight of 32,000 was detected in the active fraction. A partial peptide map was established, and degenerate DNA primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse polymerase chain reaction was used for obtaining the full-length gene. The S. uberis plasminogen-activator gene (skc) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In a protein sequence comparison, the **cloned** activator showed an identity of approximately 26% to the **streptokinases** isolated from Streptococcus equisimilis and Streptococcus pyogenes. The activator from S. uberis lacked the C-terminal domain possessed by the **streptokinase** from **S . equisimilis**. This is apparently a general feature of the **streptokinases** of this species. Sequencing of the skc gene from 3 of these strains indicated that the protein sequence of the protein is highly conserved within the species. (32 ref)

L6 ANSWER 6 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 97:555242 SCISEARCH
THE GENUINE ARTICLE: XL484
TITLE: The LppC gene of Streptococcus equisimilis encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from Haemophilus influenzae
AUTHOR: Gase K; Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H (Reprint)
CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, WINZERLAER STR 10, D-07745 JENA, GERMANY (Reprint); UNIV JENA, INST MOL BIOL, D-07745 JENA,

GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0300-8584.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 39
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing, and analysis of a novel chromosomal gene of Streptococcus equisimilis strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or **expressed** from plasmid pLPP2 in Escherichia coli was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of **S. equisimilis** and with the outer membrane of E. coli JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in S. pyogenes, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from Haemophilus influenzae to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of E. coli for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of **S. equisimilis** could grow on hemin in iron-limited medium. These results, together with findings indicating that **S. equisimilis** H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L6 ANSWER 7 OF 29 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 96:44932 LIFESCI
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus equisimilis
AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.
CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany
SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411.
ISSN: 0021-9193.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; G
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus equisimilis H46A. This gene, originally called rel, here termed rel sub( )**S. equisimilis**, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly **expressed** rel sub( )**S**. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA

gene. We verified that the Rel sub( )**S. equisimilis** protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )**S. equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )**S. equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( ) **S. equisimilis** gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel sub( )**S. equisimilis** in **S. equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L6 ANSWER 8 OF 29 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 96200111 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8631718
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus equisimilis.
AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 19970203
Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus equisimilis H46A. This gene, originally called rel, here termed relS. equisimilis, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly **expressed** rel **S. equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel **S. equisimilis** protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel **S. equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel **S. equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel **S. equisimilis** gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel **S. equisimilis** in **S. equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L6 ANSWER 9 OF 29 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 96396845 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8803948
TITLE: Structural dissection and functional analysis of the

complex promoter of the **streptokinase** gene from Streptococcus equisimilis H46A.
AUTHOR: Grafe S; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Medical microbiology and immunology, (1996 May) 185 (1) 11-7.
Journal code: 0314524. ISSN: 0300-8584.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970131

AB The overlapping tandem promoters of the **streptokinase** gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from Photinus pyralis to analyze the contribution of the different sequence elements to promoter activity in Escherichia coli and the homologous Streptococcus equisimilis strain H46A. In E. coli, virtually the entire promoter activity derived from the upstream promoter P1. In **S. equisimilis**, luc **expression** increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem promoters presumably function by mutual contributary action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong S. pneumoniae and Bacillus subtilis promoters.

L6 ANSWER 10 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:433656 HCAPLUS
DOCUMENT NUMBER: 133:27355
TITLE: **Cloning** and **expression** of Streptococcus H46 **streptokinase** gene
INVENTOR(S): Cho, Jung-Myong; Park, Yong-U.
PATENT ASSIGNEE(S): LG Chemical Co., Ltd., S. Korea
SOURCE: Repub. Korea, No pp. given
CODEN: KRXXFC
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 9512901	B1	19951023	KR 1992-17406	19920924

PRIORITY APPLN. INFO.: KR 1992-17406 19920924

AB The **cloning** of **streptokinase** gene of Streptococcus H46 consists of PCR with primers and **cloning** the gene into the PstI-NdeI site of plasmid ptrp322H-HGH (KFCC 10067) to get ptrpH-SK (ATCC 68884). The DNA sequence of Streptococcus H46 **streptokinase** has 92.2-98.8% homol. to SKC, SKG, and SKA. Streptococcus H46 is also designated **S. equisimilis** ATCC 35556.

L6 ANSWER 11 OF 29 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 95342169 MEDLINE

DOCUMENT NUMBER: PubMed ID: 7616967
TITLE: Complex transcriptional control of the **streptokinase** gene of Streptococcus equisimilis H46A.
AUTHOR: Gase K; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19950905
Entered Medline: 19950822

AB On the Streptococcus equisimilis H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (skc) and a leucine-rich protein (lrp), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of skc' and lrp' to 'lacZ from Escherichia coli. The major transcriptional start sites, in both **S. equisimilis** and E. coli, mapped 22 bases upstream of the ATG start site of lrp (G), and 24 and 32 bases upstream of the translational initiation codon of skc (A and G, respectively), indicating the existence of two overlapping canonical skc promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were **cloned** in E. coli on a vector containing a 1.1 kb fragment of the **S. equisimilis** dexB gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the skc region of the homologous host. In **S. equisimilis**, skc'-'lacZ was **expressed** about 200-fold more strongly than the corresponding lrp'-'lacZ fusion. In contrast, in E. coli, the corresponding levels of **expression** differed by only about 11-fold. Deletion of the 202 bp bent region upstream of the skc and lrp core promoters caused a 13-fold decrease in skc promoter activity in **S. equisimilis** but did not alter lrp promoter strength in this host. In contrast, when studied in E. coli, this deletion did not alter the strength of the skc-double promoter and even increased by 2.4- to 3-fold the activity of the lrp promoter. This comparative promoter analysis shows that skc has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the skc promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L6 ANSWER 12 OF 29 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 95157528 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7531815
TITLE: Transcription termination of the **streptokinase** gene of Streptococcus equisimilis H46A: bidirectionality and efficiency in homologous and heterologous hosts.
AUTHOR: Steiner K; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19960129
Entered Medline: 19950316

AB In Streptococcus equisimilis H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the **streptokinase** gene, skc, and the rel-orf1 genes. These two transcription units are oriented towards each other, and under normal conditions the skc mRNA level exceeds that of the rel-orf1 genes by a factor of at least 1000. Reporter vectors based on the promoterless cat gene were constructed by transcriptional fusion of skc to cat, such that the region between the two genes contained the terminator in skc orientation or in rel-orf1 orientation. Additionally, skc and cat were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in Escherichia coli or in single copy following integration, via skc, into the **S. equisimilis** chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in E. coli. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in rel-orf1 polarity. The results suggest that, in **S. equisimilis**, transcription of both skc and rel-orf1 is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly **expressed** rel-orf1 genes.

L6 ANSWER 13 OF 29 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 96154934 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8577315
TITLE: Conservation of the organization of the **streptokinase** gene region among pathogenic streptococci.
AUTHOR: Frank C; Steiner K; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Medical microbiology and immunology, (1995 Oct) 184 (3) 139-46.
Journal code: 0314524. ISSN: 0300-8584.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X72832
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960313

AB Using ten gene-specific probes from the **cloned** and sequenced **streptokinase** gene (skc) region (8,931 bp) of Streptococcus equisimilis H46A, a human serogroup C strain, the conservation of these genes and their linkage relationships were studied by Southern hybridization in pathogenic streptococci differing taxonomically, serologically, in regard to their host range, and in the class of plasminogen activator produced. The results indicate that in S. pyogenes (strains A374, NZ131 and SF130/13) and a human group G strain (G19,908) both gene content and gene order as determined for H46A (dexB-abc-lrp-skc-orf1-rel) are preserved. The same is true of an equine

S. equisimilis isolate (87-542-W), the **streptokinase** gene of which has been shown to hybridize detectably with skc, a result at variance with that obtained previously by others. In contrast, the chromosomal DNA of three S. uberis strains (0140J, C198, C216) of bovine origin, two of which produced a plasminogen activator different from **streptokinase**, hybridized only with dexB-, abc- and rel-specific probes, and the homologues of these genes appeared to lie close to each other. The maintenance of the organization of the **streptokinase** gene region in strains differing in overall chromosomal character suggests that this gene arrangement is of selective advantage.

L6 ANSWER 14 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:186995 HCAPLUS
DOCUMENT NUMBER: 120:186995
TITLE: Inactivation of the **streptokinase** gene prevents Streptococcus equisimilis H46A from acquiring cell-associated plasmin activity in the presence of plasminogen
AUTHOR(S): Malke, Horst; Mechold, Undine; Gase, Klaus; Gerlach, Dieter
CORPORATE SOURCE: Inst. Mol. Biol., Jena Univ., Jena, D-07745, Germany
SOURCE: FEMS Microbiology Letters (1994), 116(1), 107-12
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **streptokinase** gene of **S. equisimilis** H46 was inactivated by plasmid insertion mutagenesis to study the relation between elaboration of **streptokinase** and acquisition of cell-associated plasmin activity after incubation of wild-type and mutant cells in media containing plasminogen or plasmin. H46A binds both the zymogen and active enzyme, generates surface-associated plasmin activity in the presence of plasminogen when producing **streptokinase**, and **expresses** its plasmin(ogen) receptor(s) independently of a functional **streptokinase** gene. At least part of the plasmin(ogen) binding capacity may be due to the glyceraldehyde-3-phosphate dehydrogenase type of receptor mol., as judged by the detection of the corresponding gene.

L6 ANSWER 15 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1992:441935 HCAPLUS
DOCUMENT NUMBER: 117:41935
TITLE: **Cloning** and **expression** of **streptokinase** gene of C-type Streptococcus equisimilis
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522

AU 644657	B2	19931216	AU 1991-78101		19910531
RU 2107726	C1	19980327	RU 1991-5001280		19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A	19900523
			SU 1991-5001280	A	19910717

AB The **streptokinase** (I) gene SKC-2 ,with/without signal sequence, is **cloned** from C-type **S. equisimilis** ATCC-9542 by the polymerase chain reaction method and **expressed** in Escherichia coli and yeast for com. manufacture of I. Genomic DNA of the C-type **S. equisimilis** was isolated by the standard method and amplified with primers derived from the nucleotide sequence of SKC to get I gene with/without signal sequence. **Expression** of the I gene in E. coli and Pichia pastoris MP-36 mutant were shown. The production of I with these microorganisms were ≥350 mg/L and ≥1.2 g/L, resp.

L6 ANSWER 16 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	1992:646505 HCAPLUS
DOCUMENT NUMBER:	117:246505
TITLE:	**Streptokinase** mutation affecting skc **expression** in homologous and heterologous hosts
AUTHOR(S):	Mechold, U.; Muller, J.; Malke, H.
CORPORATE SOURCE:	Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany
SOURCE:	Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8 CODEN: ZBASE2; ISSN: 0941-018X
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Mutations affecting the level of **streptokinase** gene skc **expression** and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included skc deletions, IS element insertions, and skc duplications. The deletion events, represented by mutations Δ(skc)-247 and Δ(skc)-305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the Skc signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into E. coli and P. mirabilis L-forms, substantially increased the level of Skc **expression** in either host. In E. coli, they also facilitated the export of mature Skc into the culture medium. In the gram-pos. hosts, skc **expression** was less dramatically affected; however, the proportion of Skc activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type skc. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased Skc **expression** in all hosts but, expectedly, did not affect Skc secretability. Gene skc duplication in the chromosome of the homologous producer strain, **S. equisimilis** H46A, was achieved by a single crossover event between the chromosomes and an integrateable Skc plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant **clone** designated H46SM which was plasmid-free and produced Skc at levels approx. twice as high as the wild type.

L6 ANSWER 17 OF 29 MEDLINE on STN DUPLICATE 10

ACCESSION NUMBER:	92039051 MEDLINE
DOCUMENT NUMBER:	PubMed ID: 1937032
TITLE:	Isolation, sequence and **expression** in Escherichia

coli, Bacillus subtilis and Lactococcus lactis of the DNase (streptodornase)-encoding gene from Streptococcus equisimilis H46A.
AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G
CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.
SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727; GENBANK-M59728; GENBANK-M63990; GENBANK-S61507; GENBANK-S63856; GENBANK-S63863; GENBANK-S65020; GENBANK-S65060; GENBANK-X17241
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from Streptococcus equisimilis H64A (Lancefield Group C) was constructed in Escherichia coli. **Clones** displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was **expressed** in E. coli, Bacillus subtilis and Lactococcus lactis. As observed for **S. equisimilis**, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L6 ANSWER 18 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 91:636602 SCISEARCH
THE GENUINE ARTICLE: GQ068
TITLE: ISOLATION, SEQUENCE AND **EXPRESSION** IN ESCHERICHIA-COLI, BACILLUS-SUBTILIS AND LACTOCOCCUS-LACTIS OF THE DNASE (STREPTODORNASE)-ENCODING GENE FROM STREPTOCOCCUS-EQUISIMILIS H46A
AUTHOR: WOLINOWSKA R; CEGLOWSKI P (Reprint); KOK J; VENEMA G
CORPORATE SOURCE: MED ACAD WARSAW, DEPT PHARMACEUT MICROBIOL, OCZKI 3, PL-02007 WARSAW, POLAND; UNIV GRONINGEN, INST GENET, 9700 AB GRONINGEN, NETHERLANDS
COUNTRY OF AUTHOR: POLAND; NETHERLANDS
SOURCE: GENE, (1991) Vol. 106, No. 1, pp. 115-119.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A partial library of BclI-generated chromosomal DNA fragments from Streptococcus equisimilis H64A (Lancefield Group C) was constructed in Escherichia coli. **Clones** displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was **expressed** in E. coli, Bacillus subtilis and Lactococcus lactis. As observed for **S. equisimilis**, in the heterologous Gram+ hosts, at least part of the SDC protein was secreted

into the medium.

L6 ANSWER 19 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1991:179756 HCAPLUS
DOCUMENT NUMBER: 114:179756
TITLE: Manufacture of serotype c **streptokinase** with **recombinant** Streptococcus equisimilis
INVENTOR(S): Mueller, Joerg; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.
SOURCE: Ger. (East), 12 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284898	A5	19901128	DD 1989-332866	19890921

PRIORITY APPLN. INFO.: DD 1989-332866 19890921
AB Serotype c **streptokinase** is manufactured by **S. equisimilis** transformed with a plasmid containing the **S. equisimilis** skc gene and a selectable marker, preferably the erythromycin resistance gene. The plasmid becomes incorporated into the microbial genome by recombination to double the skc gene copy number to two. Submerged cultivation of the transformant results in the enzyme being secreted into the medium in quantities .apprx.2-fold greater than those secreted by the wild-type strain.

L6 ANSWER 20 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:625400 HCAPLUS
DOCUMENT NUMBER: 113:225400
TITLE: Duplication of the **streptokinase** gene in the chromosome of Streptococcus equisimilis H46A
AUTHOR(S): Mueller, Joerg; Malke, Horst
CORPORATE SOURCE: Acad. Sci. GDR, Cent. Inst. Microbiol. Exp. Ther., Jena, DDR-6900, Ger. Dem. Rep.
SOURCE: FEMS Microbiology Letters (1990), 72(1-2), 75-8
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The erythromycin resistance plasmid pSM752 carrying the **cloned streptokinase** gene, skc, was introduced by protoplast transformation into **S. equisimilis** H46A from which skc was originally **cloned**. Cells transiently supporting the replication of pSM752 gave rise to an erythromycin-resistant **clone** designated H46SM which was plasmid free and produced **streptokinase** at levels approx. twice as high as the wild type. Southern hybridization of total cell DNA with an skc-containing probe provided evidence for the duplication of the skc gene in the H46SM chromosome. The results, which have some bearing on industrial **streptokinase** production, can be best explained by a single cross-over event between the chromosome and the plasmid in the region of shared homol. leading to the integration of pSM752 in a Campbell-like manner.

L6 ANSWER 21 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:88986 HCAPLUS
DOCUMENT NUMBER: 108:88986
TITLE: **Expression** of **streptokinase** gene of Streptococcus in Pichia pastoris
INVENTOR(S): Hagenson, Mary Jane; Stroman, David Womack
PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA
SOURCE: Eur. Pat. Appl., 27 pp.
CODEN: EPXXDW

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 248227	A1	19871209	EP 1987-106614	19870507
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
ZA 8702534	A	19871125	ZA 1987-2534	19870408
AU 8771390	A1	19871112	AU 1987-71390	19870410
AU 592862	B2	19900125		
JP 62296881	A2	19871224	JP 1987-109620	19870502
NO 8701886	A	19871109	NO 1987-1886	19870506
DK 8702335	A	19871109	DK 1987-2335	19870507
FI 8702031	A	19871109	FI 1987-2031	19870507
BR 8702337	A	19880217	BR 1987-2337	19870507
DD 257646	A5	19880622	DD 1987-302541	19870507

PRIORITY APPLN. INFO.: US 1986-860960 19860508

AB The gene for **streptokinase** of Streptococcus equisimilis is **cloned** and **expressed** in Pichia pastoris. Plasmid pHTskc25 was constructed containing the coding sequence (minus the signal sequence) for **S. equisimilis streptokinase** under the control of the alc. oxidase gene promoter of P. pastoris. P. pastoris Transformed with the plasmid and grown in MeOH-containing medium produced 16 units **streptokinase**/O.D. cells.

L6 ANSWER 22 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:455636 HCAPLUS
DOCUMENT NUMBER: 105:55636
TITLE: The **streptokinase** gene: **cloning**, sequencing and **expression** in new hosts
AUTHOR(S): Malke, Horst
CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena, Ger. Dem. Rep.
SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986), 41(7), 502-4
CODEN: ZKMEEF; ISSN: 0233-1608
DOCUMENT TYPE: Journal
LANGUAGE: German

AB The **streptokinase** (I) [9002-01-1] gene (skc) of Streptococcus equisimilis H46A was **cloned** in Escherichia coli using vector λL47. One of the **recombinant clones** was used to subclone skc in E. coli plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in E. coli; **expression** of skc was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. **Expression** of skc was attained in S. sanguis after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with **S. equisimilis**. Use of pSM752 for similar transformation of Bacillus subtilis is briefly discussed.

L6 ANSWER 23 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 11
ACCESSION NUMBER: 1987:277792 BIOSIS
DOCUMENT NUMBER: PREV198784018831; BA84:18831
TITLE: MOLECULAR **CLONING** OF **STREPTOKINASE** GENE FROM STREPTOCOCCUS-EQUISIMILIS AND ITS **EXPRESSION**

IN ESCHERICHIA-COLI.
AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN S M
CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST SCIENCE AND TECHNOLOGY KAIST , PO BOX 150 CHONGRYANG, SEOUL 131, KOREA
SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp. 391-398.
CODEN: KBCJAK. ISSN: 0368-4881.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 19 Jun 1987
Last Updated on STN: 19 Jun 1987

AB The streptococcal genomic DNA digested with Pst I was **cloned** in E. coli HB101. The overlay technique of casein/plasminogen was used to screen the **clones** for **recombinants** carrying the **streptokinase** gene. The insert size of the plasmid carrying the **streptokinase** gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of **cloned** gene, **streptokinase** was highly purified from **S. equisimilis** by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from E. coli was identical to that from **S. equisimilis**. In the E. coli cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L6 ANSWER 24 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:220084 HCAPLUS
DOCUMENT NUMBER: 104:220084
TITLE: **Expression** of the **streptokinase** gene from Streptococcus equisimilis in Bacillus subtilis
AUTHOR(S): Klessen, Christian; Malke, Horst
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Acad. Sci. GDR, Jena, 6900, Ger. Dem. Rep.
SOURCE: Journal of Basic Microbiology (1986), 26, 75-81
CODEN: JBMIEQ; ISSN: 0233-111X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The previously **cloned** and sequenced **streptokinase** [9002-01-1] gene (skc) from **S. equisimilis** H46A was inserted into plasmid vectors capable of replication in B. subtilis. The skc gene was **expressed** by use of its own transcription and translation signals which appeared to meet the stringent requirements of B. subtilis for efficient foreign gene **expression**. The secreted **streptokinase** activity began to decline toward the end of the exponential growth phase suggesting that B. subtilis exoproteases hydrolyzed and inactivated the foreign protein.

L6 ANSWER 25 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:482518 HCAPLUS
DOCUMENT NUMBER: 103:82518
TITLE: Nucleotide sequence of the **streptokinase** gene from Streptococcus aquisimilis H46A
AUTHOR(S): Malke, Horst; Roe, Bruce; Ferretti, Joseph J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA
SOURCE: Gene (1985), 34(2-3), 357-62
CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The entire nucleotide sequence of a **cloned** 2568-base-pair (bp) PstI fragment from the genome of **S. equisimilis** H46A encoding the **streptokinase** [9002-01-1] gene (skc) was determined The longest open reading frame comprises 1320 bp which code for **streptokinase**. The protein is synthesized with a 26-amino acid residue N-terminal extension having properties characteristic of a signal peptide. Comparison of the deduced amino acid sequence with the available amino acid sequence of a com. **streptokinase** reveals minor structure differences. The nucleotide sequencing of skc does not support the hypothesis that the gene has evolved by duplication and fusion, as suggested by internal 2-fold amino acid homologies of its product. Furthermore, the skc gene sequence shows no extended regions homologous to the staphylokinase gene. Upstream from the skc gene, the putative skc promoter and the ribosome-binding site sequence were identified; downstream from the coding region, inverted repeat sequences thought to function as transcription terminators were detected.

L6 ANSWER 26 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:473464 HCAPLUS
DOCUMENT NUMBER: 105:73464
TITLE: Hybridization of a **cloned** group C streptococcal **streptokinase** gene with DNA from other streptococcal species
AUTHOR(S): Huang, T. T.; Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 234-6. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The previously **cloned streptokinase** [9002-01-1] gene (skc) of Streptococcus equisimilis and 2 subfragments were used as DNA hybridization probes to determine sequence homologies with other streptococcal species. The human pathogenic streptococci of strains A, C, and G were the only strains that had a pos. correlation between the ability to produce **streptokinase** and to hybridize with the gene skc DNA probe. In conjunction with other streptococcal DNA probes, such as streptolysin O, hyaluronidase, DNase, and erythrogenic toxins, the skc probe may be of diagnostic significance in the rapid identification of human pathogenic streptococci.

L6 ANSWER 27 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:473709 HCAPLUS
DOCUMENT NUMBER: 105:73709
TITLE: **Cloning** of streptococcal genes with Streptococcus-Escherichia coli shuttle vector pSA3
AUTHOR(S): Dao, M. L.; Ferretti, J. J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both

E. coli and S. sanguis, was constructed. Chromosomal DNA from S. mutans was ligated into this plasmid and **cloned** in E. coli. Of 472 **clones** tested, 43 **clones expressed** S. mutans surface antigens. A **cloned S. equisimilis streptokinase** [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform E. coli, S. sanguis, and S. mutans, all of which **expressed** the **cloned streptokinase** gene.

L6 ANSWER 28 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:473707 HCAPLUS
DOCUMENT NUMBER: 105:73707
TITLE: **Cloned streptokinase** gene from Streptococcus equisimilis H46A
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 221-2. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The **streptokinase** [9002-01-1] gene skc of **S. equisimilis** was **cloned** in Escherichia coli with plasmid pBR322. **Expression** of gene skc was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in E. coli. **Streptokinase** was excreted by the E. coli host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of 26 amino acids.

L6 ANSWER 29 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 12
ACCESSION NUMBER: 1985:232658 BIOSIS
DOCUMENT NUMBER: PREV198579012654; BA79:12654
TITLE: **EXPRESSION** OF A **STREPTOKINASE** GENE FROM STREPTOCOCCUS-EQUISIMILIS IN STREPTOCOCCUS-SANGUIS.
AUTHOR(S): MALKE H [Reprint author]; GERLACH D; KOEHLER W; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GDR, CENTRAL INST MICROBIOLOGY EXPERIMENTAL THERAPY, DDR-69 JENA, GDR
SOURCE: Molecular and General Genetics, (1984) Vol. 196, No. 2, pp. 360-363.
CODEN: MGGEAE. ISSN: 0026-8925.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB Using **recombinant** DNA techniques, one introduced a previously **cloned streptokinase** gene from **S. equisimilis** into the Challis strain of S. sanguis (group H). The gene was **expressed** in the new host under the control of its own promoter and the gene product had biological properties identical to authentic **streptokinase**. The MW of **cloned streptokinase** (42 K [kilodalton]) as **expressed** by S. sanguis was substantially lower than that of authentic **streptokinase** (47 K). Since the **cloned streptokinase** gene encoded a 47 K mature protein, the lowered MW of S. sanguis **streptokinase** may reflect posttranslational proteolytic cleavage, which leaves the biological activity of the gene product and its serological reactivity unimpaired.

=> s inclusion (a) bod?
L7 44795 INCLUSION (A) BOD?

```
=> d his

     (FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004
L1         40597 S STREPTOKINASE?
L2           362 S "S. EQUISIMILIS"
L3           103 S L1 AND L2
L4       6513995 S CLON? OR EXPRESS? OR RECOMBINANT
L5            74 S L3 AND L4
L6            29 DUP REM L5 (45 DUPLICATES REMOVED)
L7         44795 S INCLUSION (A) BOD?

=> s l6 and l7
L8             1 L6 AND L7

=> d all

L8   ANSWER 1 OF 1  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN   2000:96119  BIOSIS
DN   PREV200000096119
TI   Two streptokinase genes are expressed with different
     solubility in Escherichia coli W3110.
AU   Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria;
     Fernandez, Julio; Paez, Rolando; Torrens, Isis
CS   Biopharmaceutical Development Division, Center for Genetic Engineering and
     Biotechnology, Havana, Cuba
SO   Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
     CODEN: BILED3. ISSN: 0141-5492.
DT   Article
LA   English
ED   Entered STN: 15 Mar 2000
     Last Updated on STN: 3 Jan 2002
AB   The streptokinase (SK) gene from S.
     equisimilis H46A (ATCC 12449) was cloned in E. coli
     W3110 under the control of the tryptophan promoter.  The
     recombinant SK, which represented 15% of total cell protein
     content, was found in the soluble fraction of disrupted cells.  The
     solubility of this SK notably differed from that of the product of the SK
     gene from S. equisimilis (ATCC 9542) which had been
     cloned in E. coli W3110 by using similar expression
     vector and cell growth conditions, and occurred in the form of
     inclusion bodies.
CC   Genetics of bacteria and viruses   31500
     Biochemistry methods - Nucleic acids, purines and pyrimidines   10052
     Biochemistry methods - Proteins, peptides and amino acids   10054
     Replication, transcription, translation   10300
     Biophysics - Molecular properties and macromolecules   10506
     Microbiological apparatus, methods and media   32000
     Food microbiology - General and miscellaneous   39008
     Enzymes - General and comparative studies: coenzymes   10802
     Metabolism - Proteins, peptides and amino acids   13012
     Morphology and cytology of bacteria   30500
     Physiology and biochemistry of bacteria   31000
IT   Major Concepts
        Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics
        (Biochemistry and Molecular Biophysics)
IT   Chemicals & Biochemicals
        amino acids; enzymes; proteins; tryptophan
IT   Miscellaneous Descriptors
        biotechnology; cell growth conditions; expression vectors;
        gene expression; promoters; tryptophan promoter
```

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ORGN Classifier
        Enterobacteriaceae   06702
     Super Taxa
        Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;
        Microorganisms
     Organism Name
        Escherichia coli: W 3110
     Taxa Notes
        Bacteria, Eubacteria, Microorganisms
ORGN Classifier
        Gram-Positive Cocci   07700
     Super Taxa
        Eubacteria; Bacteria; Microorganisms
     Organism Name
        Streptococcus equisimilis
     Taxa Notes
        Bacteria, Eubacteria, Microorganisms
RN   54-12-6Q (tryptophan)
     73-22-3Q (tryptophan)


=> s "lambdapr"
L9          42 "LAMBDAPR"

=> d his

     (FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004
L1        40597 S STREPTOKINASE?
L2          362 S "S. EQUISIMILIS"
L3          103 S L1 AND L2
L4      6513995 S CLON? OR EXPRESS? OR RECOMBINANT
L5           74 S L3 AND L4
L6           29 DUP REM L5 (45 DUPLICATES REMOVED)
L7        44795 S INCLUSION (A) BOD?
L8            1 S L6 AND L7
L9           42 S "LAMBDAPR"

=> s l2 and l9
L10           0 L2 AND L9

=> s l1 and l9
L11           0 L1 AND L9

=> s l9(w) lambdapl
L12           0 L9(W) LAMBDAPL

=> s "lambdapr-lambdapl"
L13           0 "LAMBDAPR-LAMBDAPL"

=> dup rem l9
PROCESSING COMPLETED FOR L9
L14          35 DUP REM L9 (7 DUPLICATES REMOVED)

=> d 1-10 ibib ab

L14  ANSWER 1 OF 35  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER:   2004:147086  BIOSIS
DOCUMENT NUMBER:    PREV200400151628
TITLE:              Gene expression patterns controlled by a temperature
                    sensitive promoter.
AUTHOR(S):          Chapman, Emily [Reprint Author]; Wu, Xiao-lun [Reprint
```

Author]
CORPORATE SOURCE: Physics and Astronomy, University of Pittsburgh, Pittsburgh, PA, USA
SOURCE: Biophysical Journal, (January 2004) Vol. 86, No. 1, pp. 420a. print.
Meeting Info.: 48th Annual Meeting of the Biophysical Society. Baltimore, MD, USA. February 14-18, 2004. Biophysical Society.
ISSN: 0006-3495 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Mar 2004
Last Updated on STN: 17 Mar 2004

AB Gene expression patterns are not very well understood even in simple biological systems. This was investigated using a promoter that is repressed by the CI857 protein. We cloned GFPmut3 into a vector where the expression of the protein is controlled by the PL promoter, and the transcription of the repressor is regulated by the **lambdaPR** promoter. When the temperature of a sample reaches approximately 37degreeC the repressor protein is no longer able to bind and the PL promoter can begin expression of the GFP. We used the fluorescence properties of GFP to estimate the average amount of protein expressed in a large population of E. coli, as well as the expression level of individual cells. It was found that for minimally induced cells, there is a great deal of heterogeneities between different cells similar to those observed in the well-studied pBAD system. A simple model is constructed to explain the gene expression patterns seen in the large population and in individual cells. This temperature sensitive promoter can be a tool in the study of the dynamics of a synthetic genetic network.

L14 ANSWER 2 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2004:147069 BIOSIS
DOCUMENT NUMBER: PREV200400151620
TITLE: Urea as a probe of conformational changes in transcription initiation for E. coli RNA Polymerase.
AUTHOR(S): Kontur, Wayne S. [Reprint Author]; Davis, Caroline; Saecker, Ruth [Reprint Author]; Record, M. Thomas Jr.
CORPORATE SOURCE: Chemistry, University of Wisconsin-Madison, Madison, WI, USA
SOURCE: Biophysical Journal, (January 2004) Vol. 86, No. 1, pp. 418a. print.
Meeting Info.: 48th Annual Meeting of the Biophysical Society. Baltimore, MD, USA. February 14-18, 2004. Biophysical Society.
ISSN: 0006-3495 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Mar 2004
Last Updated on STN: 17 Mar 2004

AB Binding of E. coli RNA Polymerase Holoenzyme (R) to **lambdaPR** promoter DNA (P) to form an open complex (RPo) follows a three-step mechanism involving two intermediates, I1 and I2. Footprinting studies have been used to characterize the state of the DNA in I1 and RPo, but probes are needed to assay conformational changes that take place in the protein throughout the process. Urea effects (like dCpo effects) should be a useful diagnostic of local folding or unfolding in the steps of a mechanism. The effect of urea concentration on the equilibrium constant for protein unfolding is correlated with the amount of protein surface area exposed to the solvent (dASA) upon unfolding (Myers et al:, 95; Courtenay et al., 00): m-value=RTdlnKobs/d(urea)=0.14dASA. This same analysis can be applied to a kinetic process, where the rate constant of a step is related to dASA for formation of the transition state of the step.

From studying the urea dependence of the kinetics of the association and dissociation of polymerase and promoter DNA, we have determined that all three steps exhibit characteristic urea dependences, which provide evidence for coupled folding or unfolding of the enzyme. From the urea data, which is consistent with and expands upon previous dCpo data, we obtain evidence for large-scale folding in forming I1, and especially in forming RPo from the (I1-I2) transition state. Interestingly, the m-value for the conversion of I1 to this crucial, rate-determining transition state is negative, arguing for unfolding in this step. Structural proposals for these coupled folding/unfolding events will be presented. This type of analysis can be applied to other systems to help develop a molecular picture for the mechanism of a reaction.

L14 ANSWER 3 OF 35 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2004:327257 SCISEARCH
THE GENUINE ARTICLE: 806TB
TITLE: Differential expression of human basic fibroblast growth factor in Escherichia coli: potential role of promoter
AUTHOR: Mirzahoseini H (Reprint); Mehraein F; Omidinia E; Razavi M R
CORPORATE SOURCE: Pasteur Inst Iran, Dept Biochem, Pasteur St 69, Tehran 13164, Iran (Reprint); Pasteur Inst Iran, Dept Biochem, Tehran 13164, Iran
COUNTRY OF AUTHOR: Iran
SOURCE: WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY, (MAR 2004) Vol. 20, No. 2, pp. 161-165.
Publisher: KLUWER ACADEMIC PUBL, VAN GODEWIJCKSTRAAT 30, 3311 GZ DORDRECHT, NETHERLANDS.
ISSN: 0959-3993.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 15

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Four different expression systems were developed for expression of the cDNA encoding human basic fibroblast growth factor (hbFGF), using Escherichia coli as host organism. The hbfgf structural gene was cloned into four expression vectors, pET-3a, pTrc99A, pPR37 and pKK223-3 differing only in their promoters, which were T7, trc, **lambdaPR** and tac respectively. Expression of the gene was induced by adding 0.5 mM (final concentration) of isopropyl-beta-D- thio-galactopyranoside (IPTG) for the vectors carrying T7, trc and tac promoters or by a temperature shift from 30 to 42 degreesC for the vector carrying kPR. The highest level of expression was observed in pET-1005 (a pET-3a derivative)/BL21 (DE3) system with 18.5 mg/l rhbFGF and the second high level expression was in pR37-1007 (pPR37 derivative) BL21 (DE3) system with 5 mg of rhbFGF/l. Since in the latter system a temperature shift was used for induction, 29% of the hbFGF was recovered as inclusion bodies in the insoluble cell fraction. The level of expression for the two other systems (pTrc-1006 and pKK-1008) was very low.

L14 ANSWER 4 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-05827 BIOTECHDS
TITLE: Producing recombinant virus protein R (Vpr), useful in promoting immunodeficiency virus reproduction, involves transforming a host cell with an expression vector for the protein;
recombinant protein production via plasmid expression in host cell for use in immunodeficiency virus reproduction
PATENT ASSIGNEE: ORIENTAL YEAST CO LTD; NAKAMURA T
PATENT INFO: JP 2003259881 16 Sep 2003
APPLICATION INFO: JP 2002-66938 12 Mar 2002
PRIORITY INFO: JP 2002-66938 12 Mar 2002; JP 2002-66938 12 Mar 2002
DOCUMENT TYPE: Patent
LANGUAGE: Japanese

OTHER SOURCE: WPI: 2004-026590 [03]
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) recombinant virus protein R (Vpr) protein (I) having a fully defined sequence (S1) of 96 amino acids as given in the specification, or a sequence having one or more substitutions, additions or alterations in (S1) and having biological activity, by transforming a host Escherichia coli cell (II) with an expression vector (III) containing the gene encoding (I), and culturing the transformed cell, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) the expression vector (III) useful in M1; (2) an Escherichia coli cell (II) transformed by vector (III); (3) recombinant virus protein R (I) produced by M1; and (4) promoting (M2) immunodeficiency virus reproduction in a cell, tissue or organ derived from an organism by administering (I).

BIOTECHNOLOGY - Preferred Method: In M1, transformed cell (II) is N4830-1. (III) is p **lambdaPR**-A2 having an accession number FERM P-18748. (I) does not contain another heterologous protein or peptide except Vpr protein. In M2, the cell is a chronic sustainable virus infected cell.

USE - M1 is useful for producing recombinant Vpr protein. (I) is useful in method M2 for promoting the reproduction of immunodeficiency virus in cell, tissue or organ derived from an organism (all claimed). (I) is useful for functional analysis of the Vpr protein.

ADVANTAGE - (I) is produced efficiently by (M1) at a low cost. Functional analysis showed that Vpr enhances reproduction of HIV-1 in chronic sustainable infected U1 cell. The effect of Vpr is neutralized by anti tumor necrosis factor (TNF) alpha antibodies.

EXAMPLE - Recombinant Vpr protein was produced as follows. Recombinant full-length Vpr protein was produced by the Escherichia coli expression system. The full-length sequence of Vpr was amplified by PCR. The natural Vpr gene was mutated. The acquired PCR products were digested by the restriction enzyme NcoI and PstI and coupled with the expression vector p **lambdaPR** and p **lambdaPR**-A2 was built. The expression of a Vpr gene, which contains temperature sensitivity to lambda c1857 was used as a host cell. The transformed organisms were collected. The purified transformed organisms were cultured. The recombinant Vpr protein was purified from the supernatant liquid which contains transformed microbial cells. The produced recombinant protein was analyzed by Western blotting. The results confirmed the production of 15 KDa recombinant protein. The amount of recombinant protein produced was high. The function of Vpr in the enhancement of reproduction of HIV1 in chronic sustainable virus infected cell was studied by administering the recombinant Vpr protein. The results showed that Vpr had a specific role in the enhancement of reproduction of HIV1. The effect was similar to the function of tumor necrosis factor (TNF) alpha. The effect of the Vpr was neutralized by the anti TNF alpha antibodies. (14 pages)

L14 ANSWER 5 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:500115 BIOSIS
DOCUMENT NUMBER: PREV200300502236
TITLE: Efficient biosynthetic incorporation of tryptophan and indole analogs in an integral membrane protein.
AUTHOR(S): Broos, Jaap [Reprint Author]; Gabellieri, Edi; Biemans-Oldehinkel, Esther; Strambini, Giovanni B.
CORPORATE SOURCE: Department of Biochemistry, Groningen Biomolecular Science and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 4, 9747 AG, Groningen, Netherlands J.Broos@chem.rug.nl
SOURCE: Protein Science, (September 2003) Vol. 12, No. 9, pp. 1991-2000. print.
ISSN: 0961-8368.
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 29 Oct 2003
Last Updated on STN: 29 Oct 2003

AB Biosynthetic incorporation of tryptophan (Trp) analogs such as 7-azatryptophan, 5-hydroxytryptophan, and fluorotryptophan into a protein can facilitate its structural analysis by spectroscopic techniques such as fluorescence, phosphorescence, nuclear magnetic resonance, and Fourier transform infrared. Until now, the approach has dealt primarily with soluble proteins. In this article, we demonstrate that four different Trp analogs can be very efficiently incorporated into a membrane protein as demonstrated for the mannitol transporter of Escherichia coli (EIImtl). EIImtl overexpression was under control of the **lambdaPR** promoter, and the E. coli Trp auxotroph M5219 was used as host. This strain constitutively expresses the heat labile repressor protein of the **lambdaPR** promoter. Together with the presence of the repressor gene on the EIImtl plasmid, this resulted in a tightly controlled promoter system, a prerequisite for high Trp analog incorporation. A new method for determining the analog incorporation efficiency is presented that is suitable for membrane proteins. The procedure involves fitting of the phosphorescence spectrum as a linear combination of the Trp and Trp analog contributions, taking into account the influence of the protein environment on the Trp analog spectrum. The data show that the analog content of EIImtl samples is very high (>95%). In addition, we report here that biosynthetic incorporation of Trp analogs can also be effected with less expensive indole analogs, which in vivo are converted to L-Trp analogs.

L14 ANSWER 6 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:348497 BIOSIS
DOCUMENT NUMBER: PREV200300348497
TITLE: Interplay between DnaA and SeqA proteins during regulation of bacteriophage **lambdapR** promoter activity.
AUTHOR(S): Slominska, Monika; Konopa, Grazyna; Baranska, Sylwia; Wegrzyn, Grzegorz; Wegrzyn, Alicja [Reprint Author]
CORPORATE SOURCE: Laboratory of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, University of Gdansk, Kladki 24, 80-822, Gdansk, Poland
wegrzyn@biotech.univ.gda.pl
SOURCE: Journal of Molecular Biology, (23 May 2003) Vol. 329, No. 1, pp. 59-68. print.
ISSN: 0022-2836 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jul 2003
Last Updated on STN: 30 Jul 2003

AB DnaA and SeqA proteins are main regulators (positive and negative, respectively) of the chromosome replication in Escherichia coli. Nevertheless, both these replication regulators were found recently to be also transcription factors. Interestingly, both DnaA and SeqA control activity of the bacteriophage **lambdapR** promoter by binding downstream of the transcription start site, which is unusual among prokaryotic systems. Here we asked what are functional relationships between these two transcription regulators at one promoter region. Both in vivo and in vitro studies revealed that DnaA and SeqA can activate the pR promoter independently and separately rather than in co-operation, however, increased concentrations of one of these proteins negatively influenced the transcription stimulation mediated by the second regulator. This may suggest a competition between DnaA and SeqA for binding to the pR regulatory region. The physiological significance of this DnaA and SeqA-mediated regulation of pR is demonstrated by studies on lambda plasmid DNA replication in vivo.

L14 ANSWER 7 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-07812 BIOTECHDS
TITLE: New vector comprising 2 or more genes encoding

sugar-nucleotide regenerating enzymes and one or more gene encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale; involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis

AUTHOR: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO: US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-165735 [16]

AB DERWENT ABSTRACT:

NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), is new. The genes are operably linked to a promoter.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.

WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or glycosyltransferase enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides.

BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, SusA, GalE, GluT, Ugd or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is LgtB or LgtC. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha-3-N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably **lambdaPR** promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a glycosyltransferase, where the epimerase is UDP-Gal-4-epimerase and the glycosyltransferase is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid. Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell is a yeast cell. One of the heterologous genes in integrated into the genome of the cell. The heterologous genes are

encoded within one plasmid.

USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

L14 ANSWER 8 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-02526 BIOTECHDS
TITLE: Novel isolated protein useful for identify a compound capable of inhibiting astacin metalloproteinase activity of a parasite;
recombinant metallo protease production and drug screening useful for parasite infection therapy and vaccine preparation
AUTHOR: TRIPP C A; FRANK G R; GRIEVE R B
PATENT ASSIGNEE: HESKA CORP
PATENT INFO: US 2002086974 4 Jul 2002
APPLICATION INFO: US 2001-864541 23 May 2001
PRIORITY INFO: US 2001-864541 23 May 2001; US 1994-249552 26 May 1994
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-665480 [71]
AB DERWENT ABSTRACT:

NOVELTY - An isolated protein (I) comprising a parasite astacin metalloendopeptidase protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated antibody (II) capable of selectively binding to (I); (2) a therapeutic composition (III) for protecting an animal from disease caused by a parasite, the parasite being susceptible to an inhibitor of an astacin metalloendopeptidase, the therapeutic composition comprising at least one protective compound selected from an isolated parasite astacin metalloendopeptidase protein, an anti-parasite astacin metalloendopeptidase antibody, and an inhibitor of astacin metalloendopeptidase activity identified by its ability to inhibit parasite astacin metalloendopeptidase activity of the protein; and (3) a test kit to identify a compound capable of inhibiting astacin metalloendopeptidase activity of a parasite, the test kit comprises an isolated parasite astacin metalloendopeptidase activity and a method for determining the extent of inhibition of the activity in the presence of a putative inhibitory compound.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing in a recombinant cell, transformed with a nucleic acid molecule encoding the protein to produce the protein (claimed). Preferred Protein: If (I) is administered to an animal in an effective manner, it elicits an immune response against a parasite astacin metalloendopeptidase, where the parasite is parasitic helminths, protozoan parasites, ectoparasites, nematodes, cestodes or trematodes. The parasite comprises a tissue-migrating helminth or a nematode selected from filariid (such as Dirofilaria, Acanthocheilonema, Brugia, Dipetalonema, Loa, Onchocerca, Parafilaria, Setaria, Stephanofilaria and Wuchereria filariid nematodes), ascarid, strongyle and trichostrongyle nematodes. The parasite comprises Dirofilaria immitis. (I) is encoded by a parasite nucleic acid molecule which hybridizes under stringent conditions with D. immitis astacin metalloendopeptidase gene. (I) comprises an amino acid sequence having at least 40% homology with an amino acid sequence selected from L3 nDiMPA32076 and adult nDiMPA32028. (I) comprises at least a portion of an amino acid sequence, where the portion is encoded by a nucleic acid molecule which hybridizes under stringent conditions with a nucleic acid molecules selected from nDiMPA11299, nDiMPA22126, L3 nDiMPA32292, L3 nDiMPA32076, adult nDiMPA32032, and adult nDiMPA32028. (I) comprises an extended zinc-binding domain motif. Preferred Composition: (III) further comprises at least one component selected from an excipient, adjuvant and a carrier.

ACTIVITY - Antiparasitic; Antihelminthic.

MECHANISM OF ACTION - Inhibitor of astacin metalloproteinase activity; Vaccine. No supporting data is given.

USE - (I) is useful to identify an inhibitor of astacin metalloendopeptidase activity. (III) is useful to protect an animal from disease such as heartworm infection, caused by a parasite, by administering (III) to an animal. (I) is useful to identify a compound capable of inhibiting astacin metalloproteinase activity of a parasite, by contacting (I) with a putative inhibitory compound under conditions in which, in the absence of the compound, the astacin metalloendopeptidase protein has astacin metalloendopeptidase activity, and determining if the putative inhibitory compound inhibits the activity. (All claimed). (I) is useful to treat, ameliorate and/or prevent disease, caused by parasite. (II) is useful as vaccines to passively immunize an animal to protect the animal from parasite susceptible to treatment by such antibodies. (II) is useful as reagents in assays to detect infection by parasites and as tools to recover desired proteins from a mixture of proteins and other contaminants. (II) is also useful as immunotherapeutic agents and to target cytotoxic agents to parasites to directly kill the parasites.

ADMINISTRATION - (III) is administered by subcutaneous, intradermal, intravenous, nasal, oral, transdermal or intramuscular route. (III) is administered at a dose rate of 1 microg-10 mg/kg. Vaccine is administered at a dose of 10 microg-1 mg/kg.

EXAMPLE - Recombinant molecule plambdaPRHis-nDiMPA2804, containing nucleotides from about positions 119 through 922 of nDiMPA22126 operatively linked to a **lambdaPR** transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 6 histidines was produced. Nucleic acid molecule nDiMPA2804 was ligated. The vector which was 3455 base pairs contained 1990 base pair PvII to AatII fragment from pUC19 containing the ampicillin resistance gene and Escherichia coli origin of replication, 1100 base pair BgIII to BgIII DNA fragment from vector pRK248cIts with a PvuII linker added to one end, containing the **lambdaPR** promoter, the cI857 lambda repressor gene and 22 amino acid of the cro gene regulating lytic growth, a 55 base pair BglII to XbaI segment from pGEMEX-1 which contained the T7 promoter, a 170 base pair XbaI to ECORI segment from pRSET-B which contained the T7-S10 translational enhancer, the His6 fusion, the 11 amino acid S10 leader fusion, an enterokinase cleavage site and the multiple cloning site, and a 140 base pair fragment containing synthetic translational and transcription termination signals including the T1 translation terminators in all 3 reading frames, RNA stabilization sequence from Bacillus thurengiensis crystal protein and the T2 rho-independent transcription terminator from the trpA operon. The resulting recombinant molecule denoted plambdaPRHis-nDiMPA2804 was transformed into E. coli to form recombinant cell E. coli:plambdaPRHis-nDiMPA2804. (27 pages)

L14 ANSWER 9 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-06044 BIOTECHDS
TITLE: Novel Herpesviridae thymidine kinase mutant useful for inhibiting pathogens e.g. viruses, bacteria, tumor in animals, has one or more mutations encoding amino acid substitutions upstream from the DRH nucleoside binding site; herpes simplex virus thymidine-kinase production and its encoding gene useful for gene therapy
AUTHOR: LOEB L A; BLACK M E
PATENT ASSIGNEE: UNIV WASHINGTON
PATENT INFO: US 6451571 17 Sep 2002
APPLICATION INFO: US 1999-270956 17 Mar 1999
PRIORITY INFO: US 1999-270956 17 Mar 1999; US 1994-237592 2 May 1994
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-045581 [04]
AB DERWENT ABSTRACT:

NOVELTY - Isolated Herpesviridae thymidine kinase (TK) comprising a 12 amino acid (aa) nucleoside binding region having a site 3 made up of a DRH motif and a site 4 and mutation(s), at least one of the mutations

being an aa substitution 2 or 3 aa upstream or 5 or more aa downstream from DRH motif that increases a biological activity, preferably ability of TK to phosphorylate a nucleoside analog, as compared to unmutated TK, is new.

DETAILED DESCRIPTION - The mutant TK comprises a 12 aa nucleoside binding region having a site 3 made up of a DRH motif and a site 4 and mutation(s), at least one of the mutations being an aa substitution 2 or 3 amino acids upstream from DRH motif or a methionine or tyrosine substitution 5 amino acids downstream from the DRH motif, that increases a biological activity, preferably the ability of TK to phosphorylate a nucleoside analog, as compared to unmutated TK, such that Z is lesser than: ((TKmNAp)/(TKmTp))/((TKwtNAp)/(TKwtTp)) TKmNAp = the rate of phosphorylation of a nucleoside analog by a TK mutant; TKm Tp = the rate of phosphorylation of thymidine by a TK mutant; TKwt NAp = the rate of phosphorylation of a nucleoside analog by an unmutated TK enzyme; TKwt Tp = the rate of phosphorylation of a TK enzyme by an unmutated TK enzyme; and z = at least 1.

BIOTECHNOLOGY - Preferred Mutant: TK is Varicella Zoster virus, Herpes Simplex virus type 1 or type 2 TK. and is truncated or contains an in-frame deletion. z is 1.5, 2, 2.5, 3, 3.5, 4, 4.5 or 5. The nucleoside analog is gancyclovir, acyclovir, bucyclovir, famcyclovir, pencyclovir, valacyclovir, trifluorothymidine, 1-(2-deoxy, 2-fluoro, beta-D-arabino furanosyl)-5-iodouracil, ara-A, araT, 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine or AraC. The mutant TK comprises one or more mutations, at least one of the mutations being a cysteine substitution 6 amino acids downstream from the DRH motif, or at least 5 mutations that increase a biological activity of TK as compared to unmutated TK, including a mutation to isoleucine 3 aa upstream from DRH motif, a mutation to leucine 2 aa upstream from the DRH motif, a mutation to alanine one aa upstream from the DRH motif, a mutation to tyrosine 4 aa downstream from DRH motif and a mutation to phenylalanine 5 aa downstream from DRH motif. Alternatively the mutation is at least 4 mutations including a mutation to leucine 1 or 2 aa upstream from DRH motif, a mutation to valine 4 aa upstream from DRH motif and a mutation to methionine 5 aa downstream from DRH motif; or 4 mutations including a mutation to leucine 1 aa upstream from the DRH motif, a mutation to serine 4 aa downstream, a mutation to tyrosine 5 aa downstream and a mutation to cysteine 6 aa downstream from the DRH motif.

ACTIVITY - Virucide; Antibacterial; Antiparasitic; Cytostatic; Antipsoriatic; Immunosuppressive; Antiallergic; Vasotropic; Anti-HIV; Nootropic; Neuroprotective; Keratolytic. No biological data.

MECHANISM OF ACTION - Gene therapy.

USE - TK mutants are useful for inhibiting a pathogenic agent such as viruses, bacteria, parasites, tumor cells or autoreactive immune cells in a warm-blooded animal. TK mutant is useful for inhibiting a tumor or cancer in a warm-blooded animal, for treating a variety of disease e.g., hyperkeratosis (psoriasis), prostate hypertrophy, hyperthyroidism, endocrinopathies, autoimmune diseases, allergies, restenosis, viral diseases such as acquired immunodeficiency syndrome (AIDS) hepatitis (HCV or HBV), intracellular parasitic diseases, and to correct aberrant expression of a gene within a cell, or to replace a specific gene which is defective in proper expression, e.g. including adenosine deaminase deficiency, and Alzheimer's diseases. The mutants are utilized as a conditionally lethal marker for homologous recombination.

ADMINISTRATION - Administered by intraarticular, intracranial, intradermal, intramuscular, intraocular, intraperitoneal, intrathecal, intravenous, subcutaneous, or even directly into a tumor. Dosage details not given.

EXAMPLE - A 52-mer oligonucleotide with a wild-type thymidine kinase (tk) sequence and a 56-mer that contained degenerate nucleotides spanning from codon 165-175 of the tk gene were synthesized. Chimeric vectors pMDC (which produces an inactive TK gene product) and pMCC (which produces wild-type TK) were produced from plasmids pHETK1 and pHETK2 which

contained a HSV-1 tk structural gene, and were derivatives of pBR322. Plasmid pHETK2 contained lambdaPL and **lambdaPR** promoters, ampR, and the c1857 temperature sensitive repressor, where pHETK1 contained all the above except the lambdaPL promoter. To construct pMDC and pMCC, a dummy vector, designated pKTPD was first constructed using oligonucleotides (O1) and (O2), which were phosphorylated and then annealed. pHETK2 was digested with SstI and KpnI restriction endonucleases, and the large fragment isolated. Two picomoles of the large fragment was ligated with 6 pmol of the double-stranded oligonucleotide. The resultant double-stranded circular DNA product (designated pKTPD) was used to transform competent Escherichia coli KY895 cells. pHETK1 and pKTPD were then utilized to construct a new chimeric dummy vector, designated pMDC that produced an inactive tk gene product. Another chimeric vector, pMCC, containing the wild-type tk gene was similarly constructed by ligating the larger fragment from pHETK1 with the smaller fragment of pHETK2. pMCC produces active wild-type TK. A library containing 20% random nucleotide sequences was constructed. A 52-mer oligo containing wild-type sequences was hybridized to a 56-mer oligo which contained degenerate sequences spanning codons 165-175. The hybrid was extended with the Klenow fragment of E. coli DNA polymerase I to produce a complete double-stranded DNA product. The Klenow fragment generated double-stranded DNA was then subjected to polymerase chain reaction (PCR) amplification and the amplified DNA was digested with KpnI and SacI. The digested double-stranded oligonucleotide containing the random sequence was again purified and ligated to the KpnI/SacI digested large fragment of pMDC and used to transform competent E. coli KY895 by electroporation. An aliquot of each transformation solution was spread onto Luria Bertani (LB)-agar medium containing 50 micrograms/ml of 5'-fluorodeoxyuridine, 2 micrograms/ml of thymidine, 20 micrograms/ml of carbenicillin to determined total number of transformants. Selection for active TK clones was performed on TK selection medium that contained 50 micrograms/ml of carbenicillin, 10 micrograms/ml of 5' fluorodeoxyuridine, 2 micrograms/ml of thymidine, 20 micrograms/ml of uridine, 2% BBL peptone, 0.5% NaCl, 0.2% glucose, and 0.8% Gel-Rite. Colonies on carbenicillin medium was incubated at 37degreesC for 14-16 hours, whereas inoculated TK selection medium was incubated at 37degreesC for 24 hours. From a total of 52000 transformants that grew on carbenicillin medium, 190 were able to complement E. coli KY895 for TK function. CCCCTCGAGCGCGGTAC (O1) CGCGCTCGAGGGGAGCT (O2)(63 pages)

L14 ANSWER 10 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:43915 BIOSIS
DOCUMENT NUMBER: PREV200300043915
TITLE: Multiple mechanisms of transcription inhibition by ppGpp at the **lambdapR** promoter.
AUTHOR(S): Potrykus, Katarzyna; Wegrzyn, Grzegorz; Hernandez, V. James [Reprint Author]
CORPORATE SOURCE: Dept. of Microbiology, State University of New York, Buffalo, NY, 14214, USA
vjh@buffalo.edu
SOURCE: Journal of Biological Chemistry, (November 15 2002) Vol. 277, No. 46, pp. 43785-43791. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Jan 2003
Last Updated on STN: 15 Jan 2003

AB General stress conditions in bacterial cells cause a global cellular response called the stringent response. The first event in this control is production of large amounts of a regulatory nucleotide, guanosine-3',5'-(bis)pyrophospahte (ppGpp). It was proposed recently that ppGpp acts by decreasing stability of open complexes at promoters that make short-lived open complexes, e.g. the rRNA promoters. However, here we report that the bacteriophage **lambdapR** promoter, which forms

long-lived open complexes, is inhibited by ppGpp in vitro as observed in vivo. We performed a systematic investigation of the ppGpp-specific inhibition of transcription initiation at **lambdapR** and found that ppGpp does decrease stability of open complexes at **lambdapR** , but only slightly. Likewise the equilbrium binding constant and rate of open complex formation by RNA polymerase at **lambdapR** are only slightly affected by ppGpp. The major effect of ppGpp-mediated inhibition is to decrease the rate of promoter escape. We conclude that ppGpp-mediated inhibition of transcription initiation is not restricted to promoters that make short-lived open complexes. Rather we conclude that the initial catalytic step of transcript formation is affected by ppGpp, specifically formation of the first phosphodiester bond is inhibited by ppGpp at **lambdapR**.

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004
L1         40597 S STREPTOKINASE?
L2           362 S "S. EQUISIMILIS"
L3           103 S L1 AND L2
L4       6513995 S CLON? OR EXPRESS? OR RECOMBINANT
L5            74 S L3 AND L4
L6            29 DUP REM L5 (45 DUPLICATES REMOVED)
L7         44795 S INCLUSION (A) BOD?
L8             1 S L6 AND L7
L9            42 S "LAMBDAPR"
L10            0 S L2 AND L9
L11            0 S L1 AND L9
L12            0 S L9(W) LAMBDAPL
L13            0 S "LAMBDAPR-LAMBDAPL"
L14           35 DUP REM L9 (7 DUPLICATES REMOVED)

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E2             1     KUPPUSAMY KAVITHA/AU
E3            40 --> KUPPUSAMY M/AU
E4             2     KUPPUSAMY M R/AU
E5             1     KUPPUSAMY MUSAVAN/AU
E6             2     KUPPUSAMY N/AU
E7             2     KUPPUSAMY NALLAGOUNDER/AU
E8           447     KUPPUSAMY P/AU
E9             1     KUPPUSAMY PARIANNAN/AU
E10            1     KUPPUSAMY PERIANNAM/AU
E11          244     KUPPUSAMY PERIANNAN/AU
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=> s e3-e6
L15           45 ("KUPPUSAMY M"/AU OR "KUPPUSAMY M R"/AU OR "KUPPUSAMY MUSAVAN"/A
                 U OR "KUPPUSAMY N"/AU)

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E2             1     MOSUTO AGATIELLO L/AU
E3             0 --> MOSUVAN M/AU
E4            12     MOSUWAN L/AU
E5             5     MOSUWE E/AU
E6             1     MOSUWUN L/AU
E7             1     MOSVASSAGHI A/AU
E8             1     MOSVICHEV I F/AU
E9             1     MOSVICK J/AU
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E12           1     MOSVSICHOFF F/AU

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E2            1     MOSUTO AGATIELLO L/AU
E3            0 --> MOSUVAN K/AU
E4           12     MOSUWAN L/AU
E5            5     MOSUWE E/AU
E6            1     MOSUWUN L/AU
E7            1     MOSVASSAGHI A/AU
E8            1     MOSVICHEV I F/AU
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E10          80     MOSVOLD J/AU
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E12           1     MOSVSICHOFF F/AU

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E3            0 --> VELLIMEDU S/AU
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E7            1     VELLIN J F/AU
E8            4     VELLIN P/AU
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E10           1     VELLINES JOHN B/AU
E11           1     VELLINES R P/AU
E12           2     VELLING A/AU

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E2            4     LAHIRI ROMA/AU
E3         1594 --> LAHIRI S/AU
E4            2     LAHIRI S */AU
E5            8     LAHIRI S A/AU
E6           16     LAHIRI S B/AU
E7          563     LAHIRI S C/AU
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E10          17     LAHIRI S D/AU
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E5            1     KRISHNA E M GOPALA/AU
E6            6     KRISHNA E MURALI/AU
E7            1     KRISHNA E V/AU
E8            1     KRISHNA EN R/AU
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E11          20     KRISHNA G A/AU
E12           2     KRISHNA G AMAR/AU

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L17          18 ("KRISHNA E"/AU OR "KRISHNA E M"/AU OR "KRISHNA E M GOPALA"/AU
                OR "KRISHNA E MURALI"/AU OR "KRISHNA E V"/AU)

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L13            0 S "LAMBDAPR-LAMBDAPL"
L14           35 DUP REM L9 (7 DUPLICATES REMOVED)
                 E KUPPUSAMY M/AU
L15           45 S E3-E6
                 E MOSUVAN M/AU
                 E MOSUVAN K/AU
                 E VELLIMEDU S/AU
                 E LAHIRI S/AU
L16         1594 S E3
                 E KRISHNA E/AU
L17           18 S E3-E7

=> s l16 or l17
L18         1612 L16 OR L17

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L19            0 L1 AND L18

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                 E MOSUVAN M/AU
                 E MOSUVAN K/AU
                 E VELLIMEDU S/AU
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		E	LAHIRI S/AU
L16	1594	S	E3
		E	KRISHNA E/AU
L17	18	S	E3-E7
L18	1612	S	L16 OR L17
L19	0	S	L1 AND L18